Mechanistic modelling of tesaglitazar pharmacokinetic data in subjects with various degrees of renal function – evidence of interconversion

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Tesaglitazar, is predominantly metabolized (to an acyl glucuronide of the parent compound) and 20% of given dose is found unchanged in the urine.
- Acyl glucuronides are known to be unstable and can become hydrolysed back to parent compound, a phenomena called interconversion.

WHAT THIS STUDY ADDS

- A likely mechanism (interconversion) for the cause of the increased exposure of tesaglitazar in subjects with impaired renal function.
- A possible modelling framework to evaluate interconversion without dosing of the metabolite based on the simultaneous analysis of plasma and urine data from a group of subjects with varying renal function.
- A mechanistic understanding of the pharmacokinetic properties of tesaglitazar and its metabolite.

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AIMS

To develop a mechanistic pharmacokinetic (PK) model for tesaglitazar and its metabolite (an acyl glucuronide) following oral administration of tesaglitazar to subjects with varying renal function, and derive an explanation for the increased plasma exposure of tesaglitazar in subjects with impaired renal function.

METHODS

Data were from a 6-week study in subjects with renal insufficiency and matched controls undergoing repeated oral dosing with tesaglitazar ($n = 41$). Compartmental population PK modelling was employed to describe the PK of tesaglitazar and its metabolite, in plasma and urine, simultaneously. Two hypotheses were tested to investigate the increased exposure of tesaglitazar in subjects with renal functional impairment: tesaglitazar metabolism is correlated with renal function, or metabolite elimination is reduced in renal insufficiency, leading to increased hydrolysis (interconversion) to the parent compound via biliary circulation.

RESULTS

The hypothesis for interconversion was best supported by the data. The population PK model included first-order absorption, two-compartment disposition and separate renal (0.027 l h^{-1}) and metabolic (1.9 l h^{-1}) clearances for tesaglitazar. The model for the metabolite; one-compartment disposition with renal (saturable, $V_{\max} = 0.19 \mu\text{mol l}^{-1}$ and $K_m = 0.04 \mu\text{mol l}^{-1}$) and nonrenal clearances (1.2 l h^{-1}), biliary secretion (12 h^{-1}) to the gut, where interconversion and reabsorption (0.8 h^{-1}) of tesaglitazar occurred.

CONCLUSION

A mechanistic population PK model for tesaglitazar and its metabolite was developed in subjects with varying degrees of renal insufficiency. The model and data give insight into the likely mechanism (interconversion) of the increased tesaglitazar exposure in renally impaired subjects, and separate elimination and interconversion processes without dosing of the metabolite.

Introduction

Tesaglitazar is a dual peroxisome proliferator-activated receptor α/γ agonist previously in clinical development for the treatment of Type 2 diabetes. Clinical development of tesaglitazar was discontinued in May 2006 when Phase III studies indicated that the benefit:risk profile was unlikely to give an advantage over other currently available therapies. In an absorption, disposition, metabolism and excretion (ADME) study in healthy male subjects who were given either oral or intravenous single doses, tesaglitazar was found to be completely and rapidly absorbed [1]. Tesaglitazar was a low clearance compound with a small volume of distribution resulting in an elimination half-life of approximately 45 h. In the same study it was reported that tesaglitazar was mainly metabolized by UGT1A3 and UGT2B7, and most radioactivity (91%) was found in the urine, predominantly as the acyl glucuronide of tesaglitazar. The molecular structure of tesaglitazar and its metabolite is found in Figure 1. About 20% of the dose was recovered unchanged in the urine and plasma protein binding of tesaglitazar was high, approximately 99.9%. Furthermore, it was reported that tubular secretion was likely to contribute to renal clearance of tesaglitazar.

In the present study, where tesaglitazar pharmacokinetics (PK) was evaluated in subjects with various degrees of renal function, exposure to tesaglitazar was approximately three times higher in subjects with severe renal functional impairment than in age- and sex-matched controls with normal renal function. This increase in exposure was greater than expected, as the ADME study result showed that tesaglitazar is predominately metabolized and only 20% is renally cleared. A plausible explanation could be that the metabolism of tesaglitazar was reduced in subjects with impaired renal function (IRF). This could occur if, in subjects with IRF, tesaglitazar was metabolized

by renal function-dependent kidney enzymes, or if the tesaglitazar hepatic enzymes were downregulated, or if accumulation of inhibitors, predominantly in the renally impaired, reduced the metabolism of tesaglitazar. Another explanation could be that the lower renal clearance of the acyl glucuronide in renally impaired subjects leads to accumulation of the metabolite, which is then hydrolysed back to the parent compound, i.e. interconversion.

Vree *et al.* have shown that the kidneys can carry out conjugation reactions such as acyl glucuronidation, N-glucuronidation, and glycation and recognized that IRF not only affects renal excretion but may also affect renal metabolism [2]. Decreased hepatic metabolism has been observed in subjects with IRF, and suggested mechanisms have been downregulation of protein expression of specific isozymes or negative effect of circulation inhibitors [3]. Interconversion has been reported for a number of compounds forming acyl glucuronides, e.g. clofibric acid [4, 5], furosemide [6] and nonsteroidal anti-inflammatory drugs (NSAIDs) [7]. The interconversion of the acyl glucuronide is primarily thought to occur via biliary secretion of the metabolite and hydrolysis to parent compound by β -glucuronidases [8] in the gut, followed by reabsorption. No data have yet been reported (to our knowledge) of this type of interconversion clearance in man, since it is stipulated that it would require intravenous administration of the acyl glucuronide to solve the clearance equations [8]. Thus, studies in man report only apparent clearances for the aglycone, ignoring the reversibility of the metabolism.

The aim of the present study was to develop a mechanistic population PK model of tesaglitazar in subjects with varying renal function, using a modelling approach to investigate the mechanism for the increased plasma exposure of tesaglitazar in subjects with IRF.

Methods

Study design and population

This was an open, stratified two-centre study (SH-SBC-0007). Since this was the first time tesaglitazar had been given to subjects with reduced renal function, the study was initiated with a pilot study (Part I), in which six subjects with moderate [glomerular filtration rate (GFR) 31–50 ml min⁻¹ 1.73 m⁻²] or severe (GFR 10–30 ml min⁻¹ 1.73 m⁻²) renal insufficiency received one 0.5-mg tesaglitazar tablet once daily for 7 days. A pre-study visit (14 days prior to first dose) was included, where all subjects underwent routine screening for eligibility. Patients returned to the clinic on study days 1, 2, 7, 8 and at follow up (on day 28 $\pm$ 2).

In the full study (Part II), 23 patients with mild (GFR 51–80 ml min⁻¹ 1.73 m⁻²), moderate or severe renal insufficiency were enrolled. No subjects with IRF received dialysis. Renal function was assessed using creatinine clearance [9] calculated from S-creatinine, at the pre-entry visit, or

Figure 1

Chemical structure of tesaglitazar and its acyl glucuronide

using a valid measurement of GFR made within 6 months of the pre-entry visit. Eighteen subjects with normal renal function, matched for age and sex with the subjects with renal functional impairment, were also included in the study. All subjects received daily doses of tesaglitazar 1 mg for 42 ± 3 days. At a prestudy visit (14 days prior to first dose) all subjects underwent routine screening for eligibility. For exploratory purposes, probenecid was administered to four subjects in the control group in order to investigate the likelihood of a drug–drug interaction with tesaglitazar. The first oral probenecid dose (500 mg) was given in the evening the day before start of tesaglitazar treatment, the second dose at start of tesaglitazar dosing and the third, on the same day, in the evening. The study was conducted according to the principles of the Declaration of Helsinki and in accordance with the Guideline of Good Clinical Practice and was approved by independent ethics committees. Signed informed consent was received from all subjects.

Data

In Part I, plasma samples for determination of tesaglitazar and its metabolite were drawn during the first and last day, at predose, 1, 4 and 24 h postdose. In Part II, plasma samples were drawn at day 1 and 42 ± 3 after start of treatment at predose, 1, 2, 4, 12 and 24 h postdose. Trough samples were collected on days 14 and 28. Four additional samples were obtained on day 3 ± 1 , 6 ± 1 , 9 ± 1 and 21 ± 2 after last dose intake. Actual dosing and plasma sampling times were used in the analysis. For determination of tesaglitazar and its metabolite in urine, urine was collected in the intervals: 0–6, 6–12, 12–24 h on day 1, and during 0–24 h on day 42 ± 3 . Protocol times were used for the urine data. Blood samples were drawn at 1 h postdose on day 42 ± 3 for determination of plasma protein binding of tesaglitazar. During the study (days 1 and 42), plasma iohexol clearance (CL_{iohexol}) was determined and used as a measure of renal function. Additional information, demographics (sex, age and body weight), s-albumin, tesaglitazar free fraction (f_u) and probenecid treatment were included as covariate information.

Bioanalytical methods

Concentrations of tesaglitazar and its acyl glucuronide were determined in plasma using previously described validated reversed-phase liquid chromatography and mass spectrometry [10]. A similar method was used for determination of these analytes in urine. For tesaglitazar, lower and upper limits of quantification were 3 and 5000 nmol l⁻¹, in both plasma and urine. In plasma, the relative coefficient of variation (RCV) ranged between 0.5 and 5.2% over the linear tesaglitazar concentration range. Corresponding values in urine were 0.7–1.6%. Accuracy was 102.2% (range 101.2–104.4%) and 97.6% (95.4–99.7%) in plasma and urine, respectively. For the acyl glucuronide, the lower and upper limits of quantification were 10 and

4000 nmol l⁻¹. In plasma and urine, two standards were used (low and high range). In plasma, the RCV was 3.1–12.1% (low) and 1.8–9.8% (high); corresponding values in urine were 4.2–7.5% (low) and 3.8–13.1% (high). The accuracy was 100.6% (92.2–110.9%) and 100.9% (98.6–102.4%) in plasma and urine, respectively. Plasma concentration samples below the lower limit of quantification were omitted from the PK analysis.

The plasma protein binding of tesaglitazar was determined by ultrafiltration. The centrifuge (Hettich Zentrifugen ROTAFIXA/KS) used was tempered to 37°C and run for 1 h at 2600 r.p.m. The plasma samples were filtered at a relative centrifugal force 1200 *g* using a Centrifree-filter at 37°C for 20 min and the samples were analysed as described above.

Data analysis

The plasma concentration and urine data of tesaglitazar and the acyl glucuronide were simultaneously modelled with NONMEM version VI [11] using first-order conditional estimation method with interaction and the ADVAN6 (general nonlinear kinetics) subroutine. Three significant digits were requested and the tolerance of the differential equation solver was set to 5.

The postprocessor Xpose 3.1 [12] was used for model diagnostic purposes and for exploration of possible covariate relationships. Xpose runs in a Splus environment (Insightful Corporation, Seattle, WA, USA; version 2000 for Windows). Splus was also used for data manipulation, exploratory analysis and graphics.

Pharmacokinetic modelling

The basic structure of the model was parameterized as follows: for tesaglitazar, a two-compartment model with renal clearance (CL_{rt}), metabolic clearance (CL_{mt}), central volume of distribution (V_{cl}), peripheral volume of distribution (V_{pt}), intercompartment clearance (Q_{t}) and absorption rate constant (k_a); for the acyl glucuronide, a one-compartment model with renal clearance (CL_{rm}), nonrenal clearance (CL_{nr}) and central volume of distribution (V_{cm}). Since the oral absorption of tesaglitazar is known to be complete, the absolute bioavailability (F) was set to 1 [1].

Two hypotheses for the cause of the increased tesaglitazar exposure in subjects with IRF were tested: hypothesis A, where the metabolism of tesaglitazar was assumed to be correlated with the renal function and was incorporated by a correlation between CL_{iohexol} and the metabolic clearance of tesaglitazar; and hypothesis B, where the increased tesaglitazar exposure was due to interconversion between the acyl glucuronide and tesaglitazar. An additional compartment was incorporated to mimic biliary excretion of the acyl glucuronide into the gut, and thereafter interconversion and reabsorption of tesaglitazar into the central compartment of the parent drug. These processes were modelled as rate constants, k_{bm} and k_{int} , respectively. The two hypotheses were tested one at a time and were com-

Figure 2

Structural model overview including the two hypotheses

pared by differences in objective function value (OFV) and goodness of fit (GOF). Figure 2 gives an overview of the model structure, including the two hypotheses.

The interindividual variability (IIV) was assumed to be log-normally distributed. Plasma and urine data of tesaglitazar and the acyl glucuronide were untransformed and residual variability models tested were additive, proportional or a combination of both.

Covariate analysis

After the final structural model was established, potential covariate effects were evaluated. The covariates were: sex, age, body weight, renal function assessed by $CL_{iohexol}$, tesaglitazar free fraction (f_u), concomitant probenecid use, and S-albumin.

Covariates were evaluated by stepwise forward and backward inclusion/deletion, where covariates were forward included in to the model at the $P < 0.05$ level, and backwards deleted at $P < 0.01$. For two nested models, the difference in OFV (minus twice the log likelihood) is approximately χ^2 distributed. A difference in OFV of >3.84 (one degree of freedom) is thus significant at the 5% level, provided than an appropriate estimation method is used [13]. Corresponding values are 6.83 for $P < 0.01$.

Model assessment

A posterior predictive check [14] was performed to assess the final population PK model. One thousand datasets were simulated from the original dataset using final parameters estimates. The distribution of simulated plasma and urine data of tesaglitazar and the acyl glucuronide were compared with the observed values of the original dataset by both visual and numeric predictive assessments (VPC and NPC), and presented by subgroup (IRF vs. control subjects). The plasma data were evaluated

by graphical comparisons between the model predicted median and 95%-prediction interval (95% PI), and the observed median and all individual observations over time. For the urine data, the predictive check included numerical assessment between median predicted amounts and 95% PI, and median observed amounts and ranges at steady state (day 42).

Results

PK data from 41 individuals were available for analysis, including both Part I and II. Subject characteristics are presented in Table 1. The number of plasma concentration–time data for tesaglitazar and the acyl glucuronide were 707 and 323, respectively. The number of urine observations for tesaglitazar and metabolite were 163 and 163, respectively. One subject discontinued the study on day 28 after start of treatment, but data collected during the study period were included in the analysis. Median (and range) of tesaglitazar fraction unbound at day 42 was 0.11% (0.08–0.2) and 0.09% (0.06–0.12) for subjects with IRF and controls, respectively. Fraction unbound data were missing for three individuals; for these, the median value (0.1%) was imputed.

Pharmacokinetic analysis

Of the two hypotheses tested, the model incorporating interconversion was best supported by the data, with good fits to plasma and urine data for tesaglitazar and its metabolite. The difference in OFV between the two hypotheses was 217 ($P < 0.0001$), in favour of interconversion. Metabolic clearance of tesaglitazar was the major elimination pathway for tesaglitazar, where the renal route only represented approximately 1% of the total clearance (1.9 and 0.027 $l h^{-1}$ for CL_{mt} and CL_{rt} , respectively). Central and peripheral volumes of distribution were small, 2.1 and 5.1 l , respectively.

There were three elimination pathways incorporated for the acyl glucuronide: renal, nonrenal and biliary excretion. The renal route was found to be nonlinear and was best characterized by a Michaelis–Menten function with inhibition of the acyl glucuronide on its own renal elimination. The parameters estimated for the nonlinear renal elimination were: maximum elimination rate, V_{max} (0.19 $\mu mol h^{-1}$) and the metabolite concentration at half the maximum elimination rate, K_m (0.04 $\mu mol l^{-1}$). This corresponds to a renal clearance of 2.6 $l h^{-1}$ for an individual with a renal function of 76 $ml min^{-1} 1.73 m^{-2}$ and a metabolite plasma concentration of 0.03 $\mu mol l^{-1}$. Nonrenal clearance for the metabolite was 1.2 $l h^{-1}$. The biliary secretion rate of the acyl glucuronide to the gut was fast (11.7 h^{-1}), where the processes of hydrolysis of the acyl glucuronide and reabsorption of tesaglitazar were slower, 0.79 h^{-1} . The central volume of distribution of the metabolite was 8.5 l . Due to limited data during the absorption phase of tesaglitazar, the absorption rate constant was

Table 1

Subject characteristics

	Sex Male/female	Age (years)	Body weight (kg)	GFR* (ml min ⁻¹ 1.73 m ⁻²)	S-creatinine (μmol l ⁻¹)	S-albumin (g l ⁻¹)
Subjects with renal insufficiency (n = 23)						
Median	17/6	55	84	32	208	40
(range)		(34–78)	(62–107)	(16–94)	(107–316)	(35–46)
Healthy controls (n = 18)						
Median	10/8	53	75	90	71	40
(range)		(41–73)	(60–97)	(75–120)	(52–91)	(35–43)

*Assessed by plasma iohexol clearance.

Table 2

Parameter estimates and associated relative standard errors (RSE) from the final population PK model of tesaglitazar and the acyl glucuronide

PK parameters (unit)	Parameter estimate (RSE, %)	Interindividual variability, CV %	
		Nonparametric estimate [†]	Parametric estimate (RSE, %)
Structural model for tesaglitazar			
k_a (h ⁻¹)	1.5 (fixed)	n.e.	n.e.
CL _{mt} [*] (l h ⁻¹)	1.91 (8.2)	18	18 (34)
CL _{rt} [†] (l h ⁻¹)	0.027 (4.8)	13	20 (27)
Q_t (l h ⁻¹)	0.22 (13)	n.e.	n.e.
V_{ct} [‡] (l)	2.1 (8.1)	31	39 (27)
V_{pt} [§] (l)	5.1 (3.2)	8	16 (25)
Structural model for the acyl glucuronide			
V_{max} [†] (μmol h ⁻¹)	0.188 (23)	n.e.	n.e.
K_m (μmol l ⁻¹)	0.041 (29)	36	37 (32)
CL _{nrm} (l h ⁻¹)	1.2 (11)	47	54 (21)
k_{bm} (h ⁻¹)	11.7 (5.9)	n.e.	n.e.
k_{int} (h ⁻¹)	0.79 (7.3)	n.e.	n.e.
V_{cm} (l)	8.5 (17)	n.e.	n.e.
Significant covariate effects (multiplicative covariate models)			
f_u on CL _{mt} [§] (%/unit f_u)	555 (23)		
GFR on CL _{rt} [%/(ml min ⁻¹ 1.73 m ⁻²)]	0.99 (7.7)		
Gender on CL _{rt} (% difference in female vs. male)	-31 (17)		
Probenecid on CL _{rt} and V_{max} (% difference during probenecid intake)	-75 (3.9)		
Body weight on V_{ct} and V_{pt} (% kg ⁻¹)	0.94 (33)		
Residual error estimates (proportional models)			
Tesaglitazar in plasma (%)	9.7 (7.9)		
Tesaglitazar in urine (%)	37 (6.1)		
Metabolite in plasma (%)	18 (5.9)		
Metabolite in urine (%)	26 (9.8)		

*Centred on a subject with a tesaglitazar f_u of 0.1%. †Centred on a male subject with a glomerular filtration rate (GFR) of 76 ml min⁻¹ 1.73 m⁻², and not on concomitant probenecid use. ‡Centred on a subject with a body weight of 80 kg. § f_u varied between 0.06 and 0.2%, hence mean CL_{mt} varies from 1.5 to 3.0 l h⁻¹. ¶Estimated correlations (expressed as r) from the nonparametric variance–covariance matrix outside the absolute value of 0.4 were: CL_{mt} – V_{pt} = 0.68, CL_{mt} – V_{ct} = -0.43, CL_{mt} – CL_{nm} = -0.63, CL_{rt} – V_{ct} = -0.44, K_m – V_{ct} = -0.46 and CL_{nm} – V_{pt} = 0.47. Expected mean values of the interindividual variance estimates were: CL_{rt} = 0.0846, CL_{mt} = 0.19, K_m = 0.00093, V_{ct} = 0.0778, V_{pt} = 0.0592 and CL_{nm} = 0.232. n.e., not estimated; RSE, relative standard error (expressed as a percentage); CV%, coefficient of variation (expressed as a percentage).

fixed to 1.5 h⁻¹, which was based on prior PK knowledge of tesaglitazar. The population PK parameters with their standard error and interindividual variability of tesaglitazar and the acyl glucuronide are presented in Table 2. All parameters were estimated with reasonable precision, with relative standard errors (expressed as percentage) ranged between 3.2 and 34%. Several covariate relationships were found to be significant. Renal function, assessed with $CL_{iohexol}$, was incorporated on the renal elimination parameters for tesaglitazar and the acyl glucuronide prior to start

of the covariate analysis. For CL_{rt} , a linear slope intercept model with $CL_{iohexol}$ best described the data. Impact of renal function on metabolite renal elimination was incorporated as a direct linear relationship between V_{max} and $CL_{iohexol}$, normalized to the median observed renal function (76 ml min⁻¹ 1.73 m⁻²). A sex difference was found on CL_{rt} , where women had about 31% lower mean clearance estimates. A linear relationship between tesaglitazar free fraction and CL_{mt} was found to be significant, where CL_{mt} increased with increasing free fraction. The four subjects

Figure 3

(A,B) Visual predictive check of tesaglitazar in plasma. The bold solid and dotted lines represent observed and model predicted median. The grey area within the dotted lines is the model 95% prediction interval. The circles are the observations. (A) Subjects with renal insufficiency. (B) Control subjects

receiving three oral doses of probenecid had approximately 75% decreases in tesaglitazar renal clearance and V_{max} of the acyl glucuronide. No other covariate parameter relationships were identified.

The final population PK model was evaluated by visual and numerical predictive checks. The model adequately described the plasma and urine data of tesaglitazar and the acyl glucuronide, with no substantial bias observed for any of the analytes or matrices. However, the predictive checks show that the model had a general tendency to overpredict the observed variability. Therefore, the non-parametric method (as implemented in NONMEM VI) was used to quantify the variance–covariance matrix for the interindividual parameters [15], which resulted in an improved predictive performance of the model. Figures 3 and 4 and Table 3 present the visual and numerical predictive assessments of the model after including the nonparametric method.

Figure 4

(A,B) Visual predictive check of the acyl glucuronide in plasma. The bold solid and dotted lines represent observed and model predicted median. The grey area within the dotted lines is the model 95% prediction interval. The circles are the observations. The thin, solid horizontal line is the lower limit of quantification ($0.01 \mu\text{mol l}^{-1}$). (A) Subjects with renal insufficiency. (B) Control subjects

Discussion

This population analysis simultaneously investigated the PK properties of tesaglitazar and its metabolite (an acyl glucuronide of tesaglitazar) in plasma and urine in subjects with varying degrees of renal function. The main task was to derive a mechanism-based model that could explain the finding of approximately two-to-three times higher plasma exposure of tesaglitazar in subjects with IRF compared with healthy controls, given the observation that tesaglitazar is predominantly metabolized and only 20% of tesaglitazar is found unchanged in the urine. The result of the analysis demonstrates that the most likely explanation is that, in patients with IRF, the decreased renal elimination of the acyl glucuronide causes an increase of metabolite in plasma, which leads to increased amounts of metabolite undergoing interconversion and subsequently an accumulation of tesaglitazar. This phenomenon, also called

Table 3

Numerical predicted check for the amounts of tesaglitazar and the acyl glucuronide in urine (24-h samples) at steady state (day 42)

	Subjects with IRF Observed	Simulated	Control subjects Observed	Simulated
Tesaglitazar in urine (μmol)				
Median	0.42	0.52	0.54	0.54
Range (obs.) 95% PI (sim.)	0.18–0.97	0.13–1.4	0.38–0.79	0.13–1.2
Acyl glucuronide in urine (mmol)				
Median	0.82	0.80	1.4	1.4
Range (obs.) 95% PI (sim.)	0.21–1.8	0.26–1.9	0.54–1.7	0.62–2.4

futile cycle, has been described earlier for compounds forming acyl glucuronides, such as clofibric acid [4, 5, 16].

In man, no data have yet been presented, to our knowledge, for this type of interconversion process, since it would require intravenous administration of the acyl glucuronide to solve the clearance equations. Thus, studies in man report only apparent clearances for the aglycone, ignoring the reversibility of the metabolism. In the present study oral doses of tesaglitazar were given to subjects with varying degrees of renal function, and the plasma and urine data of tesaglitazar and its metabolite were analysed simultaneously. This enabled separation of the elimination and interconversion processes, as the ratio between tesaglitazar and the acyl glucuronide in plasma varies with varying renal functions. Hinderling has shown that the absolute bioavailability can be determined from oral data only, when assessing plasma and urine PK in subjects with different renal functions, which is in principle a similar approach to that suggested in the present analysis [17]. Further, simulation and re-estimation of the interconversion model given the study design and final parameter estimates showed that the model is both structurally and numerically identifiable.

The interconversion of the acyl glucuronide to tesaglitazar was implemented to occur in the gut, via biliary secretion of the metabolite, and thereafter hydrolysis to tesaglitazar and reabsorption back into circulation. The main reason for this model structure is that the acyl glucuronide of tesaglitazar has been found to be stable in plasma (<10% degraded after 6.5 h in plasma; unpublished data). In addition, many anionic drugs (such as NSAIDs) and their conjugated metabolites are metabolized in the liver, with subsequent excretion in urine and bile [18].

The acyl glucuronide of tesaglitazar has been shown to be substrate for the two efflux transporters MRP-2 and BCRP, indicating active secretion of the metabolite into the bile (unpublished data). In the ADME study of tesaglitazar, the mean recovery of radioactivity in faeces was approximately 8% after intravenous administration of ¹⁴C-labelled tesaglitazar, which also supports enterohepatic excretion and elimination. Identification, using mass spectrometry, revealed that the predominant portion found in faeces was

tesaglitazar [1]. This finding is in agreement with the notion that many glucuronides, following biliary excretion, may become substrates for the microfloral β-glucuronidases of the gastrointestinal tract and hydrolysed back to parent compound [19]. After hydrolysis, the liberated parent can be reabsorbed and an interconversion cycle is complete, which is likely for tesaglitazar since the absorption properties are fast with complete bioavailability.

Thus, the interconversion is suggested to occur through biliary secretion, hydrolysis in the gut and thereafter reabsorption. However, the model can not distinguish if the interconversion takes place in other tissues, but the supportive data back up the proposed model structure. When incorporating interconversion in plasma, as opposed to via biliary secretion, this model is still highly favourable compared with the hypotheses where the metabolism of tesaglitazar is reduced in subjects with IRF.

The first hypothesis tested was that the metabolism of tesaglitazar was impaired in subjects with renal insufficiency. This would be possible, for example, if the conjugation occurred in the kidneys and the metabolism decreased with decreasing renal function. An *in vitro* study, where tesaglitazar was incubated with either liver or kidney microsomes, showed that the metabolic capacity was higher in the liver, compared with the kidney (scaling of the *in vitro* data suggest that <2% of the metabolic clearance is due to renal metabolism *in vivo*, unpublished data), and this *in vitro* study also supports the rejection of the first hypothesis. Other possible mechanisms for reduced metabolism in subjects with IRF could be downregulation of hepatic enzymes or circulating inhibitors. As the model structure included interconversion of the metabolite, the clearance parameter estimates are likely to be closer to their true values, as the reversibility of the metabolism is incorporated. The renal clearance estimate of tesaglitazar (0.027 l h⁻¹ for a subject with a renal function of 76 ml min⁻¹ 1.73 m⁻²) is in agreement with the result from the ADME study (0.03 l h⁻¹). However, the total clearance reported from that study was 0.16 l h⁻¹, and is truly an apparent estimate as the impact of the interconversion was ignored. In the present analysis, the metabolic

clearance was estimated to be 1.9 l h^{-1} , much higher than the earlier reported apparent total clearance of tesaglitazar. This indicates that the interconversion of the acyl glucuronide to tesaglitazar plays an important role in the distribution and elimination of tesaglitazar.

In subjects with IRF, the impact of low renal function on its renal clearance is dual, since the low renal function increases the plasma levels of the acyl glucuronide and subsequently increases the inhibition rate of its own renal elimination due to the saturable renal elimination. K_m was estimated to be $0.04 \mu\text{mol l}^{-1}$, which is within the observed range of plasma acyl glucuronide concentrations (0.01 – $0.1 \mu\text{mol l}^{-1}$). The nonlinear renal elimination of the acyl glucuronide is determined primarily by the urine data; once this was accounted for, no signs of other nonlinear elimination routes were identified, including biliary secretion. The impaired renal elimination of the acyl glucuronide will lead to increased numbers of interconversion cycles (tesaglitazar to acyl glucuronide and back) in subjects with IRF compared with subjects with normal renal function. The first order rate constant for the biliary excretion to gut was high, where the process of interconversion and reabsorption was slower (0.8 h^{-1}) and similar to the absorption rate constant of tesaglitazar (1.5 h^{-1}). The absorption rate constant of tesaglitazar was fixed in the present analysis due to limited plasma data early after dose administration. Ericsson *et al.* have reported t_{max} to occur approximately 1 h after dose intake, which is in agreement with a predicted t_{max} of 1 h in the present model [1].

The assessment of the population PK model by numerical and visual predicted checks showed no substantial bias for any of the analytes or subgroups. When using the non-parametric option, the simulated intra- and interindividual variability were in agreement with the observed data, with modest overestimation of interindividual variability for tesaglitazar and the metabolite in plasma in the control group.

All covariates found significant were supported by basic PK principles. The renal elimination of tesaglitazar and the acyl glucuronide were correlated to renal function, with decreases in renal elimination with decreasing renal function. Concomitant probenecid use inhibited the renal elimination of tesaglitazar and its metabolite in the range of 75%, probably by inhibition of active secretion processes. A randomized, two-way crossover study in healthy male subjects, receiving single oral doses of tesaglitazar (8 mg) alone or in combination with steady-state dosing of probenecid, showed an effect of probenecid with a decrease in the amount of tesaglitazar and the acyl glucuronide in urine of about 70%, with a mean increase in plasma exposure of 2.2 and 2.9 times for tesaglitazar and the metabolite, respectively (unpublished data). These results are similar to the estimated effects from the present study, indicating the strong power of population PK modelling, since only four subjects received oral doses of

probenecid. The interaction between probenecid and tesaglitazar and its acyl glucuronide was primarily identified by the urine data. Once these interactions were accounted for, no effect of probenecid was found on any of the other elimination pathways. Tesaglitazar free fraction was found to affect the metabolic clearance of tesaglitazar, where the CL_{mt} increased with increasing free fraction, which is in agreement with tesaglitazar being a low clearance compound.

In conclusion, a mechanistic population PK model for tesaglitazar and its acyl glucuronide was developed to investigate the likely cause of the increased plasma exposure of tesaglitazar in subjects with IRF. The result suggested that the impaired renal elimination of the metabolite caused an increased amount of the acyl glucuronide to become biliary secreted, hydrolysed and reabsorbed into circulation as tesaglitazar. Thus, the use of a mechanistic modelling approach together with the present data enables separation of the elimination of the interconversion processes without intravenous dosing of the acyl glucuronide.

Competing interests

B.H. and H.E. are both employees of AstraZeneca. O.S. has been paid by AstraZeneca for consulting in the development of tesaglitazar. M.O.K. has received research funding from AstraZeneca.

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REFERENCES

- Ericsson H, Hamrén B, Bergstrand S, Elebring M, Fryklund L, Heijer M, Öhman KP. Pharmacokinetics and metabolism of tesaglitazar, a novel dual-acting peroxisome proliferator-activated receptor α/γ agonist, after a single oral and intravenous dose in humans. *Drug Metab Dispos* 2004; 32: 923–9.
- Vree TB, Hekster YA, Anderson PG. Contribution of the human kidney to the metabolic clearance of drugs. *Ann Pharmacother* 1992; 26: 1421–8.
- Dreisbach AW, Lertora JJ. The effect of chronic renal failure on hepatic drug metabolism and drug disposition. *Semin Dial* 2003; 16: 45–50.
- Gugler R, Kurten JW, Jensen CJ, Klehr U, Hartlapp J. Clofibrate disposition in renal failure and acute and chronic liver disease. *Eur J Clin Pharmacol* 1979; 15: 341–7.
- Meffin PJ, Zilm DM, Veenendaal JR. Reduced clofibric acid clearance in renal dysfunction is due to a futile cycle. *J Pharmacol Exp Ther* 1983; 227: 732–8.

- 6** Vree TB, van der Ven AJ. Clinical consequences of the biphasic elimination kinetics for the diuretic effect of furosemide and its acyl glucuronide in humans. *J Pharm Pharmacol* 1999; 51: 239–48.
- 7** Brater DC. Clinical pharmacology of NSAIDs. *J Clin Pharmacol* 1988; 28: 518–23.
- 8** Spahn-Langguth H, Benet LZ. Acyl glucuronides revisited: is the glucuronidation process a toxification as well as a detoxification mechanism? *Drug Metab Rev* 1992; 24: 5–47.
- 9** Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976; 16: 31–41.
- 10** Svennberg H, Bergh S, Stenhoff H. Factorial design for the development of automated solid-phase extraction in the 96-well format for determination of tesaglitazar, in plasma, by liquid chromatography-mass spectrometry. *J Chromatogr B Anal Technol Biomed Life Sci* 2003; 787: 231–41.
- 11** Beal SL, Sheiner LB, Boeckmann AJ. *NONMEM User's Guides*. Ellicott City, MD: Icon Development Solutions, 1989–2006.
- 12** Jonsson EN, Karlsson MO. Xpose – an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. *Comput Methods Programs Biomed* 1999; 58: 51–64.
- 13** Wahlby U, Jonsson EN, Karlsson MO. Assessment of actual significance levels for covariate effects in NONMEM. *J Pharmacokinet Pharmacodyn* 2001; 28: 231–52.
- 14** Gelman A, Carlin BJ, Stern HS, Rubin DB. *Bayesian Data Analysis*. London: Chapman & Hall, 1995.
- 15** Savic RM, Kjellsson MC, Karlsson MO. Evaluation of the nonparametric estimation method in NONMEM VI beta. *PAGE* 15 (2006) Abstr 937. Available at <http://www.page-meeting.org/?abstract=937> (last accessed: 11 February 2007).
- 16** Verbeeck RK, Branch RA, Wilkinson GR. Drug metabolites in renal failure: pharmacokinetic and clinical implications. *Clin Pharmacokinet* 1981; 6: 329–45.
- 17** Hinderling PH. Evaluation of a novel method to estimate absolute bioavailability of drugs from oral data. *Biopharm Drug Dispos* 2003; 24: 1–16.
- 18** Davies NM, Skjodt NM. Choosing the right nonsteroidal anti-inflammatory drug for the right patient: a pharmacokinetic approach. *Clin Pharmacokinet* 2000; 38: 377–92.
- 19** Ilett KF, Tee LB, Reeves PT, Minchin RF. Metabolism of drugs and other xenobiotics in the gut lumen and wall. *Pharmacol Ther* 1990; 46: 67–93.